Clinical Importance of CD200 Expression in Colorectal Liver Metastasis

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Conflicts of Interest

All authors have no conflicts of interest to declare.

Synopsis

This study demonstrated that CD200 expression is an independent prognostic factor in colorectal liver metastasis. CD200 may play a critical role and act as a potential therapeutic target for colorectal liver metastasis.

ABSTRACT

Background. Approximately 30% of patients diagnosed with colorectal cancer (CRC) develop liver metastases. We evaluated the role of CD200, a potent immunosuppressive molecule, in colorectal liver metastases (CRLM).

Methods. We examined 110 patients who underwent curative liver resection for CRLM at our institution between 2000 and 2016. Based on the results of immunohistochemical analysis, the patients were divided into high- (n = 47) and low-CD200 (n = 63) expression groups. The relationships between CD200 expression and various clinicopathological outcomes were investigated.

Results. The overall survival (OS) of the patients in the high-CD200 group was significantly worse than that in the low-CD200 group ($p = 0.009$). Multivariate analysis showed that the independent prognostic factors in CRLM were: maximum tumor size > 30 mm ($p = 0.002$), preoperative CEA level > 20 ng/ml ($p < 0.001$), primary CRC N2-3 ($p = 0.049$), and high CD200 expression ($p = 0.004$). Furthermore, CD4+, CD8+, and CD45RO+ tumor-infiltrating lymphocytes in CRLM were significantly higher in the low-CD200 group than in the high-CD200 group ($p = 0.005$, $p = 0.001$, and $p < 0.001$,

respectively). In addition, patients who had received preoperative chemotherapy had higher CD200 expression than those who had not received preoperative chemotherapy, and OS was significantly worse in the patients in the high-CD200 group who had received preoperative chemotherapy.

Conclusions. CD200 expression was an independent prognostic factor in CRLM. CD200 may play a critical role in tumor immunity in CRLM. Therefore, it can be used as a potential therapeutic target in CRLM.

Colorectal cancer (CRC) is the third and second most commonly diagnosed cancer in males and females, respectively, and the liver is the most common site of colorectal metastasis.[1] Population-based studies have shown that approximately 25–30% of patients diagnosed with CRC develop liver metastases during the course of their disease.[2] Therefore, the treatment of liver metastases is crucial in CRC. Since the development of treatment strategies such as oxaliplatin-based chemotherapy and molecular-targeted agents, several reports have indicated that combined treatment for colorectal liver metastases (CRLM) with liver resection and perioperative chemotherapy results in longer progression-free survival compared with liver resection alone.[3,4] However, there are still cases of CRLM that are difficult to treat.

Several studies have shown that primary CRC and metastatic lesions exhibit heterogeneity.[5,6] In a study by Vermaat et al., it was reported that the genetic characteristics of liver metastases and sensitivity to chemotherapy were different from those of primary CRC tumors; thus, it was proposed that the treatment choice should be based on the genetic properties of the metastatic lesion rather than those of the primary tumor.[7] Similarly, Yamamoto et al. reported that the biomarkers expressed in primary CRC and liver metastases were different, and the biomarker status in liver metastases was an independent prognostic factor.[8] Therefore, it is important to investigate the biological characteristics of CRLM to develop new therapeutic strategies that can improve the survival of patients with CRLM.

CD200 is a type I transmembrane glycoprotein related to the B7 family of costimulatory receptors. It is expressed in various cell types, including B or T lymphocytes, thymocytes, endothelial cells, and neurons. CD200 has been well-studied in hematopoietic malignancies, including lymphoma, multiple myeloma, and acute leukemia, and has been reported to affect the prognosis of these cancers.[9-12] It is overexpressed in solid tumors such as bladder cancer, breast cancer, colon cancer, melanoma, and cutaneous squamous cell carcinoma. CD200 binds to its receptor (CD200R1), which is expressed on myeloid and lymphoid cells, and plays an important role in immunosuppression and the regulation of antitumor activity,[13] such as the downregulation of macrophages,[14] induction of regulatory T cells,[15,16] and inhibition of tumor-specific T cell immunity.[17] It has been proposed that treatment strategies that with block anti-CD200 antibodies might be beneficial in patients with CD200-expressing cancers. In a phase I study in chronic lymphocytic leukemia (CLL), treatment with samalizumab, a CD200 immune checkpoint inhibitor, resulted in a reduced tumor burden in the majority of patients with advanced CLL.[18] However, CD200 seems to have a dual role in cancer development and metastasis. In the highly aggressive breast cancer animal model, CD200R agonists inhibited the metastatic growth of tumor cells that induce systemic and local inflammatory responses.[19,20] Similarly, studies have reported that the expression of CD200 inhibited tumor formation and lung metastasis in melanoma.[21,22] In contrast, CD200-positive squamous cells carcinoma showed an enhanced ability to metastasize and predicted poor prognosis.[23,24]

In another study, low levels of CD200 in rectal cancer were correlated with improved overall survival (OS) in untreated patients.[25] However, there is no study that has reported the clinical importance of CD200 expression in CRLM and the association between CD200 expression on primary CRC and CRLM. Therefore, in this study, we investigated the effect of CD200 expression on CRLM to elucidate its clinical significance

METHODS

Patients and tissue specimens

We examined the tissue specimens of 110 patients with CRLM who underwent curative liver resection for CRLM at the Department of Surgery of Nara Medical University between 2000 and 2016. In addition, the tissue specimens of 83 patients who had undergone curative colorectal resection for primary CRC at our institution were also examined. The patients' clinical data, such as demographics, tumor characteristics, perioperative chemotherapy, and follow-up data, were collected from our database and reviewed retrospectively. The clinicopathological stage was classified according to the International Union Against Cancer system. Tissue samples were obtained from the resected specimens, and each specimen was fixed in 10% phosphate-buffered formalin and embedded in paraffin. Written informed consent was obtained from all the patients before treatment, according to our institutional guidelines. The study protocol was approved by the ethics committee of Nara Medical University (approval number: 1985).

Immunohistochemistry

Formalin-fixed paraffin-embedded CRLM tissue blocks were sectioned into 5-μm slices and transferred onto glass slides. The slides were deparaffinized in three changes of xylene, rehydrated in a graded series of ethanol, and placed in distilled water. Antigen retrieval was performed by heating the tissue sections at 105°C for 20 min or 120°C for 10 min, using target retrieval solution (pH9) (DAKO, Tokyo, Japan). To block endogenous peroxidase activity, the sections were immersed in a 0.3% or 3% solution of hydrogen peroxide in absolute methanol for 20 min at room temperature and then washed thrice (5 min each) in fresh phosphate-buffered saline (PBS). Next, the sections were incubated overnight at 4°C with goat anti-human CD200 antibody (AF2724, R&D systems, Minneapolis, MN, USA) diluted 1:40 with antibody diluent (DAKO), anti-human CD45RO antibody (1:1000) (UHL1, DAKO), anti-human CD4 antibody (1:80) (4B12, DAKO), or anti-human CD8 antibody (1:100) (C8/144B, DAKO). The sections were washed thrice in PBS and incubated with Histofine Simple Stain (Nichirei Biosciences, Tokyo, Japan) or ImmPRESS Reagent (Vector Laboratories, Inc., Burlingame, USA) at 37°C for 30 min. To reveal the color of antibody staining, the tissue sections were treated with 3,3′ diaminobenzidine HRP substrate (Immpact DAB, Vector Laboratories). Subsequently, the sections were counterstained with hematoxylin, dehydrated in ethanol, cleared in xylene, and covered with cover slips.

Evaluation of immunohistochemistry

The immunohistochemical staining of CD200 was evaluated according to the intensity of the staining. For each sample, five fields with at least 200 tumor cells were randomly selected and scored, and the percentage of positively-stained tumor cells was recorded, along with the corresponding staining intensity. The staining intensity was classified into four groups: none (0 point), weak (1 point), intermediate (2 points), and strong (3 points). The positively-stained tumor cells were classified into four groups based on the percentage of cells: 0-25% (1 point), 26-50% (2 points), 51-75% (3 points), and 76-100% (4 points). The CD200 expression in each tissue sample was evaluated by adding the scores for each parameter (total score, 1-7). The specimens with scores of 1-5 were classified as having low CD200 expression, whereas those with scores of 6-7 were classified as having high CD200 expression, as described previously.[26-28] Immunohistochemical staining of CD4+, CD8+, and CD45RO+ T cells was evaluated by counting the number of tumor-infiltrating lymphocytes (TILs), as described previously.[26-28] For each sample, we selected five different areas that had the maximum number of positively-stained cells under 200× magnification. The positively-stained cells in the selected areas for each T cell marker were counted independently.

Statistical analysis

The significance of the differences in CD200 expression related to several clinicopathological variables was examined. Student's t-test was used to compare continuous variables, whereas Chi-squared test or Fisher's exact test was used to compare categorical variables between the two groups. The Kaplan-Meier method was used to estimate the probability of survival, and the corresponding significance was assessed by the log-rank test. Univariate and multivariate analyses were performed using the Cox proportional hazards model to identify significant prognostic predictors. All p values of < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

CD200 expression in CRLM and its effect on clinicopathological characteristics

We first examined the expression of CD200 in tissue samples of 110 patients with resected CRLM and 83 patients with resected CRC, by immunohistochemistry. The expression of CD200 was identified in the cytoplasm of both the primary tumor and the metastatic lesion. On the other hand, there was none or moderate expression of CD200 in normal hepatocytes or bile duct cells (Fig. 1). In case of colorectal cells, only weak expression was observed in normal endothelial cells or glandular cells. CD200 expression was almost homogeneous stained within the single metastatic tumor.

The clinicopathological characteristics of the patients are summarized in Table 1. The median age of the patients was 64.5 years (35-85 years); 65 (59.1%) were males

and 45 (40.9%) were females. To investigate the clinical importance of tumor CD200 expression in CRLM, we divided the patients into two groups: low-CD200 group (n = 63) and high-CD200 group (n = 47), according to the criteria mentioned specified in the Methods section (Fig. 1). It was observed that number of males was significantly higher in the high-CD200 group than in the low-CD200 group (p = 0.040), and the number of patients who received preoperative chemotherapy was significantly higher in the high-CD200 group than in the low-CD200 group (p = 0.034). In addition, CD200 expression was not found to be associated with age, tumor size, number of tumors, preoperative carcinoembryonic antigen (CEA) level, the primary CRC status, and *RAS* mutation status.

Effect of tumor CD200 expression on postoperative recurrence and survival

Next, we compared postoperative survival and recurrence according to the CD200 status. The median follow-up period was 41.6 months, (range, 2.5-194.3 months). The 1-, 3-, and 5-year survival rates in the low- and high-CD200 groups were 96.8%, 72.1%, and 54.1% and 89.4%, 56.8%, and 26.9%, respectively. The OS in the high-CD200 group was significantly worse than the OS in the low-CD200 group (p = 0.009), whereas there was no significant difference in recurrence free survival (RFS) between the two groups (Fig. 2). The median OS time was 4.1 years in the low-CD200 group and 2.6 years in the high-CD200 group.

Additionally, we examined the prognostic value of CD200 expression in CRLM. As shown in Table 2, univariate analysis demonstrated the following factors as significant negative prognostic factors for OS: maximum tumor size ≥ 30 mm (p = 0.027), preoperative CEA level ≥ 20 ng/ml ($p < 0.001$), primary CRC N factor of N2-3 (p = 0.008), and high CD200 expression (p = 0.009). Multivariate analysis further revealed that high CD200 expression, maximum tumor size, preoperative CEA level, and primary CRC N factor of N2-3 were independent prognostic factors. We also examined the available tissue specimens of 83 patients with primary CRC. No significant association was found between CD200 expression in primary CRC and CRLM. In addition, CD200 expression in primary CRC was not found to be correlated with OS after liver resection (data not shown).

Association of CD200 expression with tumor-infiltrating T cell subsets

We examined the correlation between tumor CD200 expression and the presence of tumor-infiltrating T cells in CRLM by immunohistochemical analysis. Results showed that the number of tumor-infiltrating CD4+, CD8+, and CD45RO+ T cells in CRLM was significantly lower in the high-CD200 group than in the low-CD200 group ($p = 0.005$, $p = 0.001$, and $p < 0.001$, respectively) (Fig. 3).

Relationship between CD200 expression and preoperative chemotherapy

Regarding the indications for preoperative chemotherapy during this study period, if the surgeon judged that all tumors could be resected within Makuuchi criteria, the patients was treated with upfront surgery regardless of the number of tumors. Patients who were deemed unresectable performed conversion surgery after chemotherapy. In this study, 52 patients had preoperative chemotherapy. Although CEA level before starting the treatment for CRLM was significantly higher in the patients with preoperative chemotherapy than those without preoperative chemotherapy, there was no significant difference in CEA level immediately before liver resection between the two groups. Other oncological factors had no differences (Supplement Table) and overall survival was similar (Supplement Fig.).

Among the 52 patients who received preoperative chemotherapy, 28 patients (53.8%) had high CD200 expression in CRLM. On the other hand, among 58 patients who did not receive preoperative chemotherapy, 19 patients (32.8%) had high CD200 expression in CRLM. The proportion of patients in the high-CD200 group was significantly higher in the patients with preoperative chemotherapy than those without preoperative chemotherapy ($p = 0.034$) (Fig. 4). Concerning long-term prognosis, there was no significant difference in OS of the patients without preoperative chemotherapy regardless of CD200 expression. However, in patients who received preoperative chemotherapy, the OS was significantly worse in the high-CD200 group than that in the low-CD200 group ($p = 0.043$). The 5-year survival rate was 21.6 % in the high-CD200 group and 54.2% in the low-CD200 group. These results showed that the patients in the

high CD200 group with preoperative chemotherapy had extremely poor prognosis. There was no significant difference in RFS between the two groups, regardless of whether or not the patients received preoperative chemotherapy (Fig. 4).

Correlation between tumor CD200 expression and recurrence pattern

Recurrence was observed in 44 patients (69.8%) in the low-CD200 group and 33 patients (70.2%) in the high-CD200 group. The recurrence after initial liver resection in the low-CD200 group was observed in the intrahepatic site in 19 patients (43.2%), the extrahepatic sites in 15 patients (34.1%), and both the intrahepatic and the extrahepatic sites in 10 patients (22.7%). Details of extrahepatic recurrence was lung in 17 patients (38.6%), peritoneum in 3 patients (6.8%), and others in 11patients (25.0%). In the high-CD200 group, the recurrence was observed in the intrahepatic site in 16 patients (48.5%), the extrahepatic sites in 9 patients (27.3%), and both the intrahepatic and the extrahepatic sites in 8 patients (24.2%). Details of extrahepatic recurrence was lung in 11 patients (33.3%), peritoneum in 4 patients (12.1%), and others in 6 patients (18.1%). The recurrence pattern between the two groups was not significantly different. Moreover, repeat liver resection was performed for 17 patients (38.6%) in the low-CD200 group and 13 patients (39.4%) in the high-CD200 group. The resection for extrahepatic metastases was performed in 8 patients (18.2%) in the low-CD200 group and 2 patients (6.1%) in the high-CD200 group. There were also no significant differences in the proportion of repeat hepatectomy or resection for extrahepatic

metastases between the two groups. Despite the fact that the pattern of recurrence or treatment for recurrence were similar between the two groups, the median survival time after recurrence was 33.6 months in the low-CD200 groups and 27.9 months in the high-CD200 group ($p = 0.048$). Focusing on intrahepatic recurrence, the median number of recurrence tumor after initial liver resection was one tumor (range, 1-10) in the low-CD200 group and three tumors (range, 1-15) in the high-CD200 group ($p = 0.003$). Moreover, in the patients of intrahepatic recurrence, multiple intrahepatic recurrence within one year after initial liver resection was reported in 12 patients (41.4%) in the low-CD200 group, and in 19 patients (79.2%) in the high-CD200 group ($p = 0.016$). In particular, among these patients, the proportion of the patients who underwent curative repeat liver resection in the high-CD200 group was lower than that in the low-CD200 group (18.8% vs. 50.0%, respectively, $p = 0.052$). Moreover, after recurrence in these patients, the OS was significantly worse in the high-CD200 group than that in the low-CD200 group ($p = 0.049$) (Fig. 5). Taken together, these results suggested that CD200 expression in CRLM might be involved in early recurrence and resectability after liver resection.

DISCUSSION

In this study, CD200 expression was observed in 42.7% of patients with CRLM. The patients in the high-CD200 group had significantly poorer prognosis than the patients in the low-CD200 group. Furthermore, high-CD200 expression was found to be

an independent risk factor for poor OS in patients who underwent CRLM resection. In contrast, CD200 expression of primary CRC was not associated with prognosis (data not shown). These data suggest that CD200 expression might have a crucial role in the progression of hepatic metastasis of CRC.

To clarify the underlying mechanism responsible for the prognostic impact of CD200 expression in CRLM, we evaluated the correlation between CD200 expression and the presence of TILs in CRLM. Several studies have shown that TILs play a significant role in the inhibition of tumor progression and disease recurrence, and thus represent a major prognostic factor in CRC and CRLM.[29-35] It is generally assumed that CD8+ TILs play important roles in the host immune defense against tumor progression.[29] Considered as memory T cells, CD45RO+ TILs can survive for many months or years and are critically important for host tumor immunity. Naito et al. first described the infiltration of colorectal cancer cell nests by CD8+ TILs as a prognostic factor in CRC.[36] In addition, Pages et al. reported the presence of CD45RO+ memory T cells in the tumor as an independent prognostic factor in CRC.[37] In this study, we found that CD200 expression was inversely correlated with not only CD4+ and CD8+ TILs levels but also with the levels of CD45RO+ memory T cells in CRLM. These findings suggest that CD200 expressed in CRLM inhibited the invasion of T cells to the tumors, thereby resulting in immune evasion.

Next, we investigated the relation between CD200 expression and chemotherapy. As shown in Figure 4, patients who received preoperative chemotherapy had higher

CD200 expression than those without preoperative chemotherapy. Furthermore, the OS was significantly worse in the high-CD200 group among the patients with preoperative chemotherapy, whereas no significant difference was observed in patients without preoperative chemotherapy. In contrast, there was no difference in RFS between the two groups, regardless of the performance of chemotherapy. These results suggested that CD200 expressed tumor remained after chemotherapy and might be resistant to chemotherapy. Based on these data, we assumed that preoperative chemotherapy might induce the expression of tumor CD200 in CRLM. Recently, CD200 has been also recognized as a cancer stem cell (CSC) as it has properties such as self-renewal, chemoresistance, and metastatic potential.[38-41] In addition, CD200 has been reported to be co-expressed with CSC markers, such as CD44 and CD133, in colon as well as prostate, breast, brain, and melanoma cancer cells.[42] Furthermore, Zhang et al. showed that CD200 expressed colorectal cancer stem cells had greater colony formation and higher invasiveness abilities.[43] In addition, Jung et al. described that in head and neck squamous cell carcinoma, tumor cells that strongly expressed CD200 grew significantly faster than those that expressed lower concentrations of CD200, after chemoradiation.[44] In summary, CD200 expression in CRLM might be induced by chemotherapy possibly on cancer stem cell, and exert high chemoresistance, thereby resulting in poor prognosis.

We also examined the recurrence pattern after liver resection for CRLM. Our results showed that there was no difference in the recurrence site after initial liver

resection between the high- and low-CD200 groups. However, the frequency of multiple intrahepatic recurrence within one year after initial liver resection was significantly higher in the high-CD200 group than in the low-CD200 group. Among these patients, repeat liver resection was more often performed in the low-CD200 group compared to that in the high-CD200 group. In addition, OS after recurrence was significantly better in the low-CD200 group than in the high-CD200 group. Previous reports have shown that repeat liver resection for CRLM is crucial for prolonging the survival.[45,46] In summary, CD200 expression might be prone to early multiple unresectable recurrence after initial hepatectomy, and was considered to have an impact on the prognosis after recurrence. These data suggest that effective antitumor immunity may be inhibited through CD200 expression in CRLM tumors, thereby contributing to intrahepatic recurrence after initial liver resection and further prognosis. However, the effect may not be reached to control systemic recurrence.

Recently, studies have highlighted the significance of the inhibitory immune pathways as therapeutic targets to strengthen anti-tumor responses and develop therapeutic strategies in cancer treatment.[47-50] Furthermore, cytotoxic T lymphocyte-associated antigen 4 or programmed cell death 1 have gained worldwide approval for the treatment of various cancers as immune checkpoint-blocking antibodies.[49] However, the clinical effects of these strategies are limited and further therapeutic targets are needed to be explored. This study demonstrated that CD200 may be used as a new therapeutic target for CRLM. In fact, the clinical trial of targeting

CD200 in hematologic cancers is currently ongoing.[18] Therefore, our study further supports the clinical application of CD200 in the treatment of CRLM. In a clinical setting, the combination of the other immune checkpoint inhibitor or conventional chemotherapy with CD200 blockade may be desirable, especially for unresectable or intractable CRLM.

This study had several limitations. First, the number of patients included in this study was relatively small. In addition, the examined samples of CRLM included only one lesion for each patient; thus, the heterogeneity of CD200 expression inside individual tumors or patients could not be explored. More importantly, information on the fundamental underlying mechanisms is lacking, which hampers our interpretation of the clinical data. Therefore, further basic as well as large-scale clinical studies are required for the clinical application of the findings of this study.

CONCLUSIONS

In this study, we have shown for the first time, to our knowledge, that CD200 expression might be related to the clinical course of CRLM. These results suggest that CD200 expression is an independent prognostic factor in CRLM and could be utilized as a potent therapeutic target for novel therapy in CRLM.

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Figure legends

Fig. 1 Representative cases of high and low CD200 expression in CRLM. High CD200 expression was detected in **(a)**, and low CD200 expression was detected in **(b)**. (original magnification, 200×)

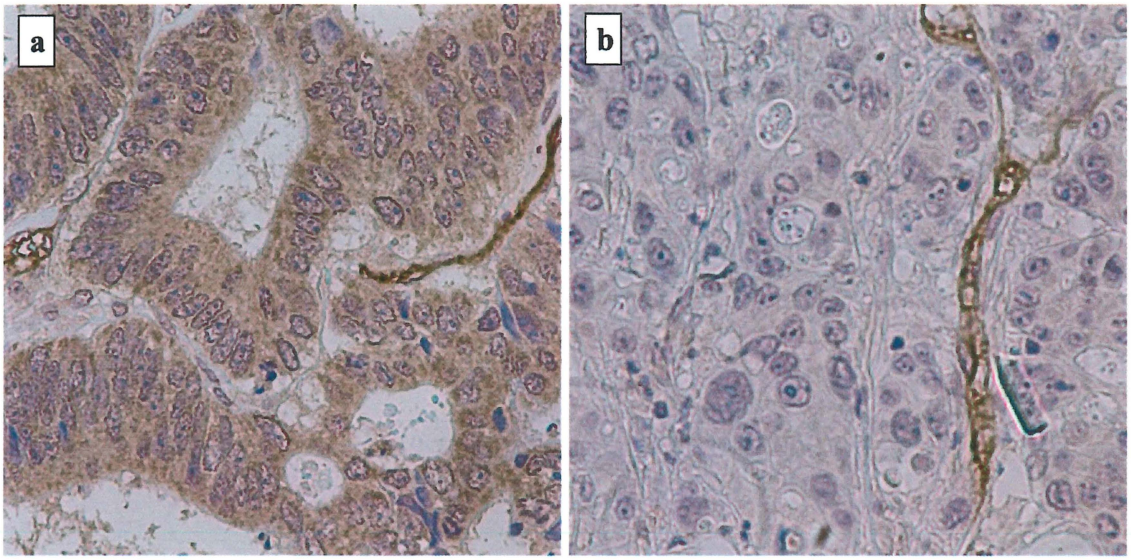
Fig. 2 Relationship between CD200 expression in CRLM and prognosis after liver resection. **(a)** Overall survival was significantly worse in the high-CD200 group than in the low-CD200 group ($p = 0.009$). **(b)** Recurrence free survival did not differ between the two groups.

Fig. 3 a, **b**, and **c** show representative images of tumor infiltrating lymphocytes (TILs) in CRLM. (original magnification, 200×) **(a)** CD4+ lymphocytes. **(b)** CD8+ lymphocytes. **(c)** CD45RO+ lymphocytes. **(d), (e)**, and **(f)** show relationship between CD200 expression and the number of TILs in CRLM. The number of CD4+ TILs (**d**), CD8+ TILs (**e**), and CD45RO+ TILs (**f**) was significantly higher in the low-CD200 group than in the high-CD200 group ($p = 0.005$, $p = 0.001$, and $p < 0.001$, respectively).

Fig. 4 Relationship between CD200 expression and preoperative chemotherapy. **(a)** In the high-CD200 group, the proportion of the patients with preoperative chemotherapy was significantly higher than those without preoperative chemotherapy (53.8% vs. 32.8%, $p = 0.034$). **(b)** In case of patients with preoperative chemotherapy, overall survival (OS) was significantly worse in the high-CD200 group than in the low-CD200 group ($p = 0.043$). **(c)** In case of patients without preoperative chemotherapy, there was no significant difference in OS between the two groups. **(d)** and **(e)** There was no significant difference in recurrence-free survival between the two groups, irrespective of whether or not they received preoperative chemotherapy.

Fig. 5 Overall survival (OS) after recurrence in patients with multiple intrahepatic metastases within one year. OS was significantly better in the low-CD200 group than in the high-CD200 group ($p = 0.049$).

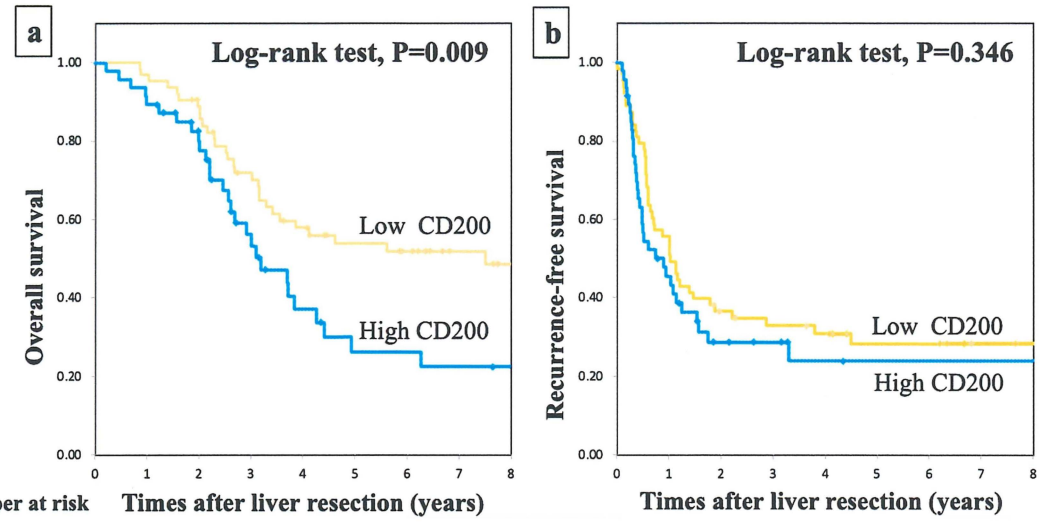
Fig. 1A, 1B



CD200 high

CD200 low

Fig. 2A, 2B



Number at risk										
Low CD200	63	56	33	26	14	63	24	16	12	7
High CD200	47	36	20	8	6	47	11	6	5	5

Fig. 3A, 3B, 3C

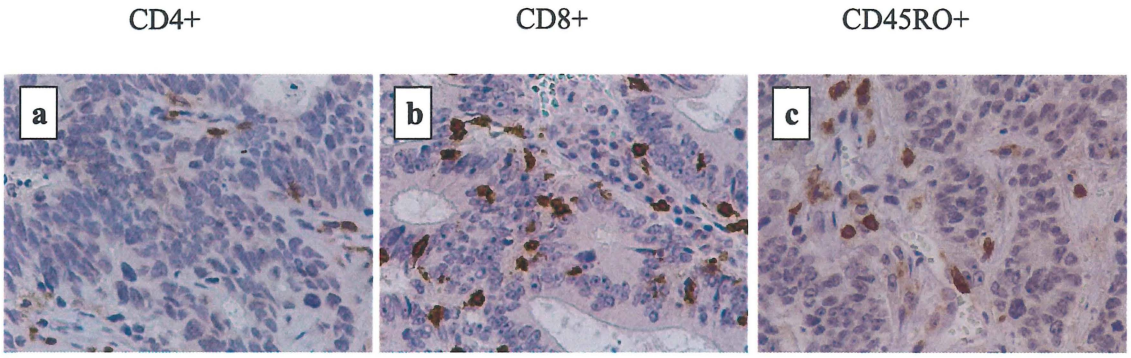


Fig. 3D, 3E, 3F

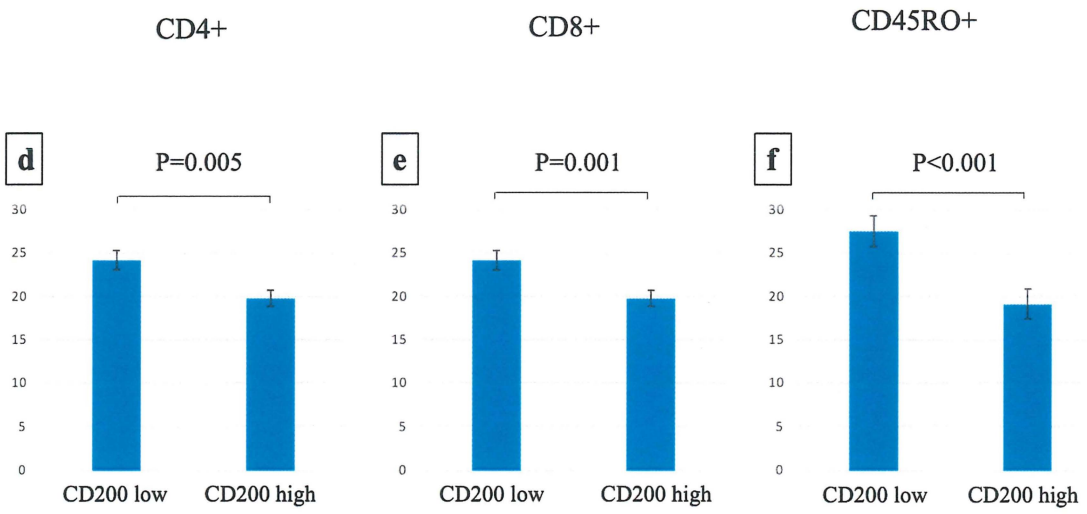
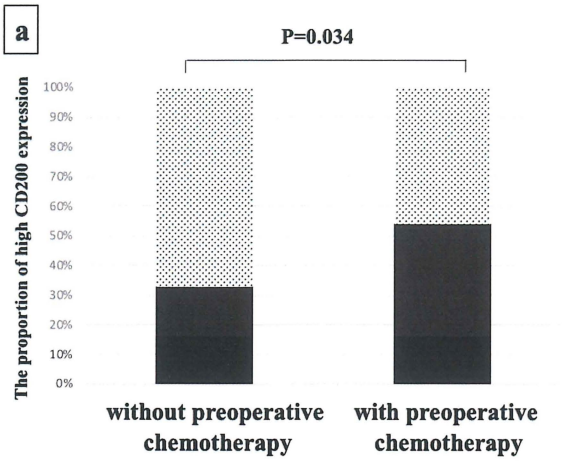
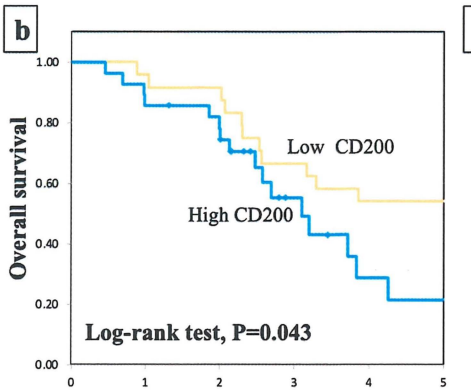


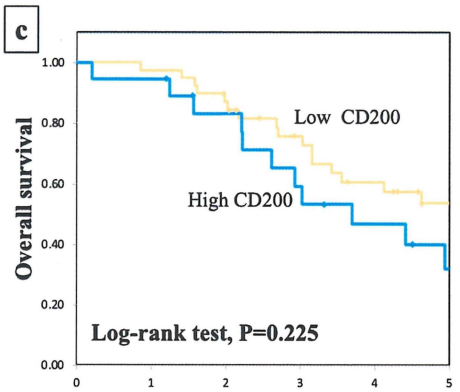
Fig. 4



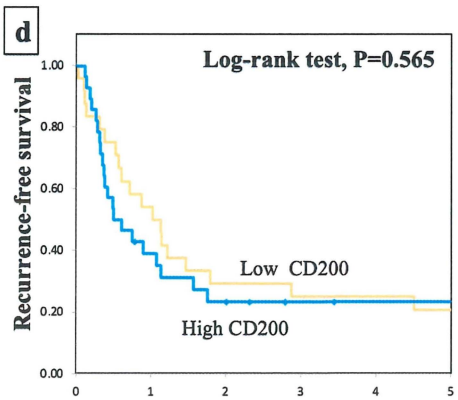
with preoperative chemotherapy

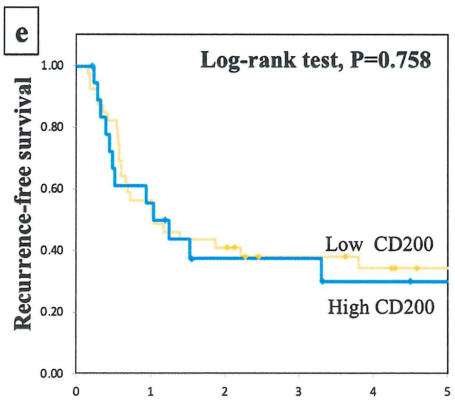
without preoperative chemotherapy





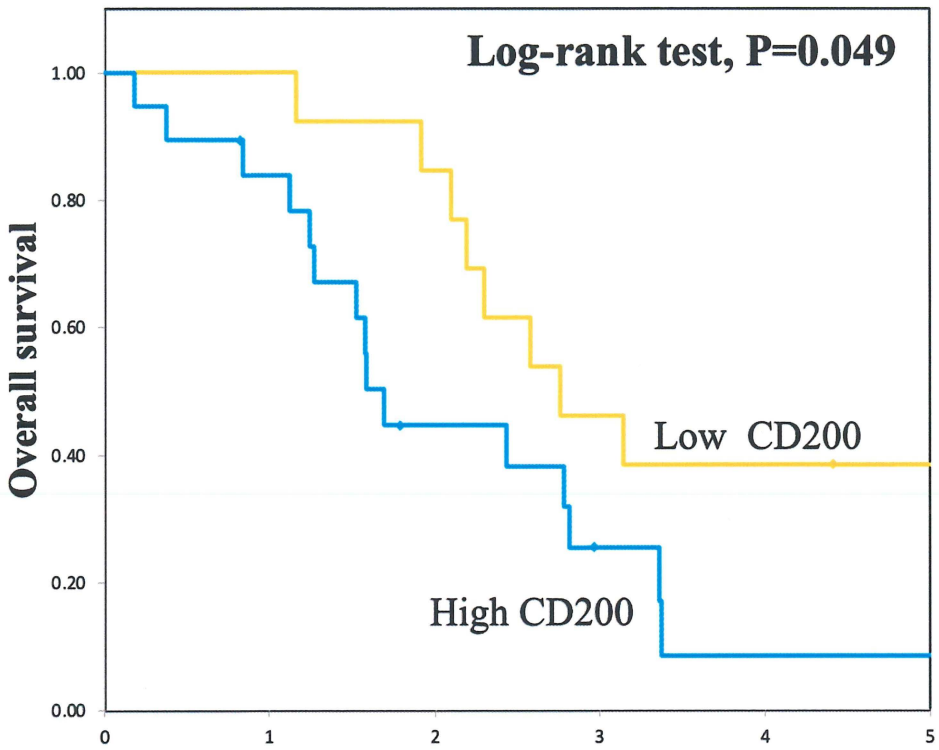
Number at risk												
Low CD200	24	23	22	17	14	14	39	38	34	26	20	14
High CD200	28	25	22	10	5	4	19	18	15	11	8	5





Number at risk												
Low CD200	24	14	8	7	7	6	39	23	17	12	10	7
High CD200	28	11	7	4	3	3	19	11	6	6	4	3

Fig. 5



Number at risk						
Low CD200	13	13	11	7	6	5
High CD200	19	16	8	4	2	2

TABLE 1 Correlation between CD200 expression and clinicopathological characteristics

	Low CD200 ($n = 63$)	High CD200 ($n = 47$)	*P value*
Age in years, Median (range)	63 (35-84)	67.5 (38-85)	0.123
Male sex, *n* (%)	32 (50.8)	33 (70.2)	0.040
CRLM status			
Presentation of liver metastases, Synchronous, *n* (%)	31 (49.2)	27 (57.4)	0.327
Maximum tumor size, mm, Median (range)	25 (8-200)	25 (10-100)	0.434
Tumor number ≥ 4, *n* (%)	15 (23.8)	15 (31.9)	0.345
Preoperative CEA level, ng/ml, Median (range)	10.7 (0.9-3391)	10.8 (1.4-1488)	1.000
Preoperative chemotherapy, *n* (%)	24 (38.1)	28 (59.6)	0.034
Primary CRC status			
T factor of primary CRC, T3-4, *n* (%)	55 (87.3)	42 (89.4)	0.914
N factor of primary CRC, N2-3, *n* (%)	18 (28.6)	12 (25.5)	0.610
Lymphatic invasion of CRC, Positive, *n* (%)	51 (81.0)	36 (76.6)	0.270
Venous invasion of primary CRC, Positive, *n* (%)	49 (77.7)	33 (70.2)	0.229
Location of primary CRC, Right, *n* (%)	15 (23.8)	13 (27.7)	0.647
Histopathological differentiation of primary CRC, well, *n* (%)	19 (30.2)	16 (34.0)	0.665
CD200 expression of primary CRC ($n = 83$)	23 (50.0)	11 (29.7)	0.062

CEA, carcinoembryonic antigen; CRC, colorectal cancer; CRLM, colorectal liver metastases

TABLE 2 Univariate and multivariate analysis of factors associated with overall survival

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P Value*	HR	95% CI	*P Value*
Age (years)						
Age ≥ 65	Referent					
Age < 65	1.115	0.665-1.868	0.680			
Gender						
Male	Referent					
Female	0.963	0.572-1.620	0.886			
Presentation of CRLM						
Synchronous	Referent					
Metachronous	0.820	0.487-1.382	0.457			
Preoperative chemotherapy						
Absent	Referent					
Present	1.352	0.790-2.315	0.271			
Postoperative chemotherapy						
Absent	Referent					
Present	0.605	0.350-1.047	0.073			
Maximum tumor size (mm)						
< 30	Referent			Referent		
≥ 30	1.187	1.020-1.383	0.027	1.309	1.103-1.553	0.002
Tumor number						
< 4	Referent					
≥ 4	1.227	0.929-1.622	0.150			
Preoperative CEA level (ng/ml)						
< 20	Referent			Referent		
≥ 20	3.014	1.799-5.050	<0.001	2.814	1.624-4.875	<0.001
Location of primary CRC						
Right	Referent					
Left	0.928	0.516-1.670	0.804			
T factor of primary CRC						
T1-2	Referent					
T3-4	1.544	0.661-3.610	0.316			
CRC N stage						
N0-1	Referent					
N2-3	2.108	1.213-3.664	0.008	1.182	1.002-3.275	0.049
Lymphatic invasion of CRC						
Positive	Referent					
Negative	1.253	0.495-3.170	0.634			
Venous invasion of primary CRC						
Positive	Referent					
Negative	1.075	0.553-2.090	0.831			
Histological differentiation of primary CRC						
Other	Referent					
Well	0.695	0.392-1.233	0.214			
RAS status						
Wild	Referent					
Mutant	0.945	0.387-2.307	0.900			
CD200 expression						
Low	Referent			Referent		
High	1.986	1.181-3.339	0.009	2.236	1.298-3.850	0.004

CEA, carcinoembryonic antigen; CI confidence interval; CRLM, colorectal liver metastases; CRC, colorectal cancer; HR, hazard ratio

Supplement TABLE Correlation between preoperative chemotherapy and oncological background

	Chemotherapy (-) (n = 58)	Chemotherapy (+) (n = 52)	*P value*
Presentation of liver metastases, Synchronous, n (%)	35 (60.3)	23 (44.2)	0.111
Maximum tumor size, mm, Median (range)	25 (8-110)	25 (10-200)	0.450
Tumor number, n, Median (range)	1.5 (1-12)	2 (1-26)	0.113
CEA level before starting treatment, ng/ml, Median (range)	9.9 (0.9-2452)	28.7 (2-3083)	0.036
CEA level before liver resection, ng/ml, Median (range)	9.9 (0.9-2452)	11.3 (1.1-3391)	0.902
T factor of primary CRC, T3-4, n (%)	52 (89.7)	45 (86.6)	0.813
N factor of primary CRC, N2-3, n (%)	16 (27.6)	14 (26.9)	0.910

Supplement FIGURE Relationship between preoperative chemotherapy and prognosis after liver resection